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Comparative Analysis of the Cardioprotective Properties of Opioid Receptor Agonists in a Rat Model of Myocardial Infarction

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Abstract

Objectives—This study was conducted to test the hypothesis that opioid receptor (OR) mediated cardioprotection is agonist-specific when administered prior to coronary artery occlusion and reperfusion in a rat model.

Methods—Anesthetized open-chest male Wistar rats were subjected to 45 minutes of left coronary artery occlusion and 2 hours of reperfusion. Opioid agonists were infused 15 minutes prior to coronary artery occlusion. Two control groups and 15 opioid treated groups were studied. Controls were infused with either saline alone (n = 16) or dimethyl sulfoxide (DMSO) plus hydroxypropyl- β -cyclodextrin in saline (n = 19). The μ selective agonist DAMGO was infused at either 150 nmol/kg (n = 15) or 1500 nmol/kg (n = 14), and Dermorphin-H was infused at 150 nmol/kg (n = 14). The δ_1 selective agonist D-Pen^{2,5} Enkephalin (DPDPE) was infused at 150 nmol/kg (n = 16) or 1500 nmol/kg (n = 14). The δ_2 selective agonists Deltorphan II (n = 16), Deltorphan-Dvariant (n = 15) and Deltorphan-E (n = 14) were infused at 150 nmol/kg. The selective κ_1 opioid agonist U-50488 was infused at 240 nmol/kg (n = 14), 1500 nmol/kg (n = 14), and 2,400 nmol/kg (n = 14). The selective κ_2 opioid agonist GR-89696 was infused at 150 nmol/kg (n = 14) and 1500 nmol/kg (n = 15). Orphanin FQ (Nociceptin), also referred to as OR Ligand1 (ORL1), was infused at 220 nmol/kg (n = 15) and 1500 nmol/kg (n = 15). The infarct size/area at risk (IS/AAR) ratio was determined after reperfusion by negative staining with patent blue violet dye. Hemodynamic parameters including heart rate, mean arterial blood pressure (MAP), and rate pressure product (RPP) were determined.

Results—Pretreatment with the δ_2 OR agonist Deltorphan II (150 nmol/kg) significantly reduced the IS/AAR ratio, while Deltorphan-Dvariant and Deltorphan-E did not exhibit an infarct sparing effect at that treatment dose. Activation of δ_1 OR by DPDPE, κ_1 OR by U-50488, κ_2 OR by U-50488, μ OR by DAMGO, Dermorphin-H, and Nociceptin had no effect on the IS/AAR ratio.

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U-50488 at 2,400 nmol/l induced a bradycardic effect. All other opioids had no effect on hemodynamic parameters at the doses tested.

Conclusions—Peripheral δ_2 OR activation by Deltorphin II induces infarct size reduction in this animal model. Agonists of μ , δ_1 , κ_1 , κ_2 , and Nociceptin receptors at the doses tested did not induce cardiac tolerance to ischemia/reperfusion injury in vivo.

Keywords

heart; ischemia; reperfusion; opioid receptors

INTRODUCTION

In 1997, it was shown that morphine could mimic the cardioprotective effect of ischemic preconditioning in vivo.¹ Schultz et al. demonstrated that morphine induced protection against infarction was mediated by δ opioid receptor (OR) stimulation.¹ The cardioprotective properties of morphine are controversial, since some investigators could not confirm its cardioprotective properties.² In 1998, in vivo data were presented that TAN-67, a selective δ_1 OR agonist, exhibited cardioprotective properties via δ_1 OR occupancy.³ The non-selective peptide δ OR agonist DADLE (D-Ala²-D-Leu⁵-enkephalin) also protected the isolated rat heart against ischemia/reperfusion via δ OR stimulation.⁴ However, DADLE did not affect the tolerance of the heart to ischemia/reperfusion injury in swine.⁵ Recently, it has been shown that the selective peptide δ_1 agonist DPDPE ([D-Pen^{2,5}]enkephalin) and the peptide δ_2 agonist Deltorphin-D variant (Tyr-D-Ala-Phe-Ala-Asp-Val-Ala-Ser-Thr-Ile-Gly-Asp-Phe-Phe-His-Ser-Ile-NH₂) (Deltorphin-D_{variant}) reduced the infarct size/area at risk (IS/AAR) ratio in rats and swine.^{5,6} Thus, published data indicate that δ_1 OR stimulation increases cardiac tolerance to ischemic and reperfusion injury. However, it is unclear whether these receptors are located in the brain or in the periphery.

While a study by Schultz et al. showed that the selective μ OR agonist DAMGO ([D-Ala²,N-Me-Phe⁴,Gly-ol⁵]enkephalin) had no effect on the IS/AAR ratio, this study used a low dose (0.1 mg/kg) of DAMGO.⁷ Accordingly, it is possible to postulate that DAMGO will exhibit cardioprotective properties at higher doses. The data on the involvement of κ ORs in the regulation of cardiac tolerance to ischemia and reperfusion are the most controversial. While at least one study has demonstrated that κ ORs play an important role in ischemic preconditioning,⁸ another demonstrated that these receptors are not involved in ischemic preconditioning.⁷ It has been reported that κ OR stimulation both in vivo^{9,10} and in vitro^{8,11,12,13} can increase the tolerance of cardiac cells to ischemia (anoxia) and reperfusion (reoxygenation) injury. However, some investigations have indicated that κ OR activation exacerbated ischemia/reperfusion damage in the isolated perfused rat heart.¹⁴ Likewise, other investigators have shown that κ OR agonists accentuated ischemic and reperfusion heart damage in vivo.^{2,5} To the best of our knowledge, no one has studied a role of opioid receptor-like 1-receptors (ORL1) in the regulation of cardiac tolerance to ischemic and reperfusion injury.

Overall, the role of opioid receptors in the regulation of cardiac tolerance to ischemia and reperfusion injury is controversial. We hypothesized that OR mediated cardioprotection may be agonist dependent. Accordingly, the specific aim of our present study was to evaluate the cardioprotective effect of selected exogenously administered μ , κ_1 , δ_1 , δ_2 , and ORL1-receptor agonists in order to further define the role of opioid receptors in the cardiac response to ischemia and reperfusion injury.

METHODS

Study Design

To determine the acute effects of opioid receptor agonists on ischemic tolerance, we used an intact rat heart model of ischemia and reperfusion. Animals were maintained and used in accordance with the guidelines of the Ethical Committee of the Institute of Cardiology of the Siberian Branch of the Russian Academy of Medical Sciences.

Animal Subjects and Preparation

Male Wistar rats weighing 250 to 300g were obtained from the colony of the Tomsk Institute of Cardiology. The animals were maintained on a 12:12-hour light-dark cycle and were given unrestricted access to food and water. Randomization was performed by alternating animals in opioid and control groups on a daily basis.

Animals were anesthetized via intraperitoneal administration of α -chloralose at a dose of 50 mg/kg. A tracheotomy was performed, and the trachea was intubated with a cannula connected to a ventilator (modified model RO-6, Kasnogvardeets, St. Petersburg, Russia). The rats were ventilated with room air supplemented with 100% O₂. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5 to 10 mm H₂O. Arterial pH, partial pressure of carbon dioxide (PCO₂), and partial pressure of oxygen (PO₂) were monitored throughout the protocol with a blood gas analyzer (Stat Profile M, Nova Biomedical Corporation, Waltham, MA) and maintained within a normal physiological range (pH 7.35 – 7.45; PCO₂ 25 – 40 mm Hg; PO₂ 80-100 mm Hg) by adjusting the respiratory rate and/or tidal volume. Body temperature was maintained at 37 °C by the use of a heating pad. The left femoral artery was cannulated for blood pressure, heart rate, and blood gas measurements. Hemodynamics, including heart rate, arterial pressure, and rate pressure product were quantified during baseline, ischemia, and reperfusion for all experimental groups. Blood pressure and electrocardiogram recordings were performed with a MP35 apparatus (Biopac Systems Inc., Goleta, CA) and an Intel computer using the applied program BSL PRO 3.7.3 (Biopac Systems Inc., Goleta, CA). The right femoral vein was cannulated for intravenous (IV) delivery of saline or drug infusion. Ischemia, reperfusion, and myocardial staining were performed according to the method of Schultz et al.¹

A left thoracotomy was performed at the fifth intercostal space, followed by a pericardiotomy and adjustment of the left atrial appendage to reveal the location of the left coronary artery. A ligature (6-0 Prolene) was passed below the left descending vein and coronary artery from the area immediately below the left atrial appendage to the right portion of the left ventricle. The ends of the suture were threaded through a polypropylene tube to form a snare, which was tightened to occlude the artery. Occlusion of the coronary artery and subsequent regional left ventricular ischemia was produced by pulling the ends of the suture taut and clamping the snare onto the epicardial surface with a hemostat. The ST segment elevation in the electrocardiogram was used to verify coronary artery occlusion. The duration of occlusion was 45 minutes. Animals were omitted from further data analysis if intractable ventricular fibrillation occurred or if severe hypotension was observed in which the experiment could not be successfully continued for the duration of the protocol. This was observed in 6% of the rats initially entered in testing.

Reperfusion of the heart was initiated by unclamping the hemostat and relieving tension on the snare, and was confirmed by visualizing a marked epicardial hyperemic response. The duration of reperfusion was 2 hours.

Study Protocol

The choice of doses of OR ligands has been guided by 1) results from our preliminary experiments with the nonselective peptide OR agonist Dalargin (H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH), which exhibited an antifibrillatory effect during coronary artery ligation at a dose of 150 nmol/kg^{15,16}, 2) our experiments with other OR ligands^{17,18}, and 3) data from other investigators^{7,9,19}. Therefore, all opioid peptides with the exception of Nociceptin (Orphanan FQ-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln), Dermorphin-H (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-Gly-Glu-Ala-Lys-Lys-Ile-NH₂), Deltorphan-D_{variant}, and Deltorphan-E (Tyr-D-Ala-Phe-Ala-Ile-Gly-Asp-Phe-Ser-Ile-NH₂) were used at a dose of 150 nmol/kg and 1500 nmol/kg.

Animals were randomly assigned to one of 15 opioid-treated groups and two control groups. Control animals received either saline, or a 20% hydroxypropyl- β -cyclodextrin solution. Dermorphin-H, Deltorphan-D_{variant}, and Deltorphan-E, not soluble in saline, were first dissolved in 0.1 ml DMSO then in 1 ml of a 20% hydroxypropyl- β -cyclodextrin solution that was used for IV administration. Deltorphan-II (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH₂), DAMGO, DPDPE, (-)-U-50488 (-)(3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl cyclohexyl) benzene-acetamide hydrochloride), Nociceptin, and GR-89696 (4-([3,4-dichlorophenyl]-acetyl)-3-(1-pyrrolidinylmethyl)-1-piper-azinecarboxylic acid methyl ester fumarate) were dissolved in 0.9% sodium chloride solution. All solutions were prepared immediately before use and given IV as a bolus dose (0.1 ml/100 g body weight) 15 minutes prior to coronary artery occlusion. All infusions were blinded to control for the subsequent determination of the IS/AAR ratio.

The μ selective agonists studied were DAMGO and Dermorphin-H. DAMGO²⁰ was infused at a dose of 0.077 mg/kg (150 nmol/kg) or 0.77 mg/kg (1500 nmol/kg). Dermorphin-H was infused at a dose of 0.23 mg/kg (150 nmol/kg).²¹

The delta-selective agonists studied were DPDPE, Deltorphan-II, Deltorphan-D_{variant}, and Deltorphan-E. The highly selective δ_1 OR agonist DPDPE^{22,23} was infused at a dose of 0.097 mg/kg (150 nmol/kg) and 0.97 mg/kg (1500 nmol/kg). The δ_2 -selective agonist Deltorphan-II²³ was infused at a dose of 0.12 mg/kg (150 nmol/kg), Deltorphan-D_{variant} at a dose of 0.3 mg/kg (150 nmol/kg), and Deltorphan-E at a dose of 0.18 mg/kg (150 nmol/kg).

The κ selective agonists were U-50488 and GR-89676.^{15,24} The κ_1 selective agonist U-50488 was infused at a dose of 0.1 mg/kg (240 nmol/kg) as recommended by Peart et al.,⁹ at 0.6 mg/kg (1500 nmol/kg),²⁴ and at 1.0 mg/kg (2400 nmol/kg).¹⁹ The κ_2 selective agonist GR-89696^{25,26} was infused at a dose of 0.08 mg/kg (150 nmol/kg) and 0.8 mg/kg (1500 nmol/kg). The endogenous ORL1, Nociceptin,²⁷ was infused at a dose of 0.4 mg (220 nmol/kg) and at 2.7 mg/kg (1500 nmol/kg). The choice of the first dose of Nociceptin was guided by results from preliminary experiments showing that this peptide at 0.4 mg/kg could delay an appearance of aconitine-induced arrhythmias.¹⁷ All infusions were blinded.

Deltorphan-II, DAMGO, DPDPE, and Nociceptin were synthesized by Multiple Peptide Systems (San Diego, CA) and by NeoMPS, Inc. (San Diego, CA). Dermorphin-H, Deltorphan-D_{variant}, and Deltorphan-E were synthesized by Bachem Inc. (King of Prussia, PA). Patent blue violet, nitrotetrazolium blue, α -chloralose, and (-)-U-50488 were obtained from Sigma-Aldrich Corporation (St. Louis, MO). Hydroxypropyl- β -cyclodextrin was purchased from Tocris Cookson (Bristol, UK). GR-89696 was synthesized by GlaxoSmithKline (Herts, UK).

Hemodynamic Parameters

Hemodynamic parameters, including heart rate, mean arterial blood pressure (MAP), and rate pressure product (RPP) were determined at baseline, following 45 minutes of ischemia, and following 2 hours of ischemia for controls and opioid-treated rats. Left ventricle weight in mg, area at risk (AAR) in mg, infarct size in mg (IS), AAR as a percentage of left ventricle weight (AAR/LV %), and IS as a percentage of AAR (IS/AAR %) were determined for all animals.

Determination of Infarct Size

After each experiment, the coronary artery was reoccluded, and the AAR was determined by negative staining. Patent blue violet dye (150 μ l, 10% w/v) was injected into the femoral vein to effectively stain the non occluded area of the left ventricle. The duration of administration was 20-30 seconds. The heart was excised, and the left ventricle was removed from the remaining tissue and subsequently cut into five thin cross-sectioned pieces. This allowed for the delineation of the normal area, which stained blue, versus the AAR, which subsequently remained pink. The AAR was excised from the non-ischemic area and the tissues were placed in separate vials and incubated for 15 minutes in a 0.1% solution of nitroterazolium blue chloride in 100 mM phosphate buffer (pH 7.4) at 37 °C. The infarcted myocardium was dissected from the AAR. IS and AAR were determined by gravimetric analysis. IS was expressed as a percentage of the AAR. Scoring of the AAR/IS ratio was done in a blinded fashion by co-investigator AK.

Data Analysis

Quantitative values are expressed as the mean $\pm$ SD. One-way analysis of variance with Newman-Keuls post hoc test was used to determine whether any significant differences existed in any parameter between groups in qualitative values. Significant differences were determined at $p < 0.05$.

RESULTS

Hemodynamics

All rats equilibrated quickly after instrumentation. Baseline levels for all rats before treatment were: heart rate, 345 ± 11 beats/min; systolic pressure, 118 ± 5 mmHg; diastolic pressure, 92 ± 2 mmHg; mean arterial blood pressure, 106 ± 2 mmHg; rate pressure product, 41 ± 2 mmHg/s/1,000.

Table 1 summarizes the hemodynamic data obtained in the experiments. No significant differences were seen between control and treatment groups for MAP. After 45 min of occlusion and 120 min of reperfusion, untreated hearts ($n = 24$) exhibited a reduction in rate pressure product (29 ± 2 mmHg/s/1,000) due to a decrease in mean arterial blood pressure. Pretreatment with U-50488 at a dose of 1 mg/kg induced a decrease in the heart rate from 325 ± 9 before injection to 284 ± 10 ($p < 0.01$) beats/min 10 min after opioid administration. Simultaneously, the rate pressure product was reduced from 32 ± 2 mmHg/s/1,000 ($p < 0.01$) without significant alteration in blood pressure. This compound exhibited a similar effect at a dose of 0.6 mg/kg but had no effect at a dose of 0.1 mg/kg. No other differences between groups were seen for hemodynamic parameters.

Infarct size

No differences were noted in the AAR/LV% between groups. Only the selective δ_2 OR agonist Deltorphin II (0.12 mg/kg) exhibited cardioprotective properties. Pretreatment with

this opioid peptide markedly reduced infarct size from 65.4% ($\pm$ 14.4%) for untreated rats to 50.6% ($\pm$ 12.4%) ($p < 0.01$, Table 2 and 3).

Activation of μ OR with DAMGO (0.077 mg/kg, 0.77 mg/kg) or Dermorphin-H (0.23 mg/kg) had no effect on the IS/AAR ratio (Table 2 and 3). Stimulation of δ_1 OR with DPDPE (0.097 mg/kg, 0.97 mg/kg) also did not alter the IS/AAR ratio. Pretreatment with the putative δ_2 OR agonists Deltorphan-D_{variant} (0.3 mg/kg) and Deltorphan-E (0.18 mg/kg) did not induce cardiac tolerance to ischemic and reperfusion injury (Table 2). The selective κ_1 OR agonist (-)-U-50488 (0.1 mg/kg, 0.6 mg/kg, 1 mg/kg) did not affect the IS/AAR ratio, and the selective κ_2 OR agonist GR-89696 (0.08 mg/kg, 0.8 mg/kg) also did not exhibit cardioprotective properties (Table 4). Finally, the endogenous agonist of ORL1 receptor Nociceptin (0.4 mg/kg, 2.7 mg/kg) did not alter the IS/AAR index (Table 2 and 3).

Analysis of the Deltorphan II results (Table 2) using power analysis and a decrease in IS/AAR ratio approaching one standard deviation from the mean indicates that the chance of a Type I error was small.

DISCUSSION

This study examined the effects of different OR agonists on the IS/AAR ratio in an in vivo rat model. It demonstrated that pretreatment with the selective δ_2 OR agonist Deltorphan-II decreased the IS/AAR ratio, and thus provided cardioprotection. This result is in agreement with our recent findings,²⁸ where we found that the cardioprotective effect of Deltorphan-II was mediated via peripheral δ_2 OR activation because cardioprotection was abolished by pretreatment with the selective δ_2 OR antagonist naltriben, and the peripherally acting OR inhibitor naloxone methiodide.²⁸ The cardioprotective effect of Deltorphan-II was also reversed by pretreatment with the nitric oxide synthase (NOS) inhibitor L-NAME, the protein kinase c (PKC) inhibitor chelerythrine, the non-selective K_{ATP} channel blocker glibenclamide, and the selective mitochondrial K_{ATP} channel inhibitor 5-hydroxydecanoate.²⁸ Maslova et al. indicated that NOS, PKC, and mitochondrial K_{ATP} channel activation play an important role in the mechanism of the infarct-reducing effect of Deltorphan-II.²⁸

The present study also demonstrated that pretreatment with the selective μ OR agonist DAMGO both at low (0.077 mg/kg) and high doses (0.77 mg/kg), and Dermorphin-H at a dose of 0.23 mg/kg, had no effect on the IS/AAR ratio. These results are in agreement with the data of Schultz et al., who also did not find an infarct-reducing effect of DAMGO at a dose of 0.1 mg/kg.⁷ It has also been shown that opioid peptides in low doses cannot cross the blood-brain barrier.^{22,29,30} In support of this, the opioid peptide Dalargin did not exhibit central effects when it was used at a dose lower than 0.5 mg/kg.³⁰ Moreover, Delay-Goyet et al. reported that only 0.038% of [³H]DAMGO crossed the blood-brain barrier after IV administration and exhibited antinociceptive properties in the mouse hotplate test only at a higher dose of 2.5 mg/kg.²² We therefore used DAMGO at doses of 0.077 and 0.77 mg/kg, and Dermorphin-H at a dose of 0.23 mg/kg. Our studies indicate that peripheral μ OR occupancy by the aforementioned agonists at doses tested does not affect cardiac tolerance to ischemia and reperfusion injury.

The selective δ_1 OR agonist DPDPE at doses of 0.097 and 0.97 mg/kg did not reduce the left ventricular infarct size when compared to the saline control. However, it has been reported that DPDPE at a dose of 2 mg/kg could reduce the IS/AAR ratio in swine.⁵ In another study, DPDPE at a dose of 3 mg/kg exhibited an infarct-reducing effect in rats.⁶ The possibility exists that the infarct reduction effects of DPDPE are dose- and species-dependent. Consequently, higher doses of DPDPE may be required to reduce infarct size in rats. Our results are also not consistent with data from another study, which reported that TAN-67, a

selective δ_1 OR agonist, exhibited cardioprotective properties in vivo via δ_1 OR occupancy.³ However, it should be noted that the study used a very high dose of TAN-67 (10 mg/kg). In addition, TAN-67 is a non-peptide δ_1 OR agonist, which most likely crosses the blood-brain barrier. It has also been recently demonstrated that intrathecal morphine infusion reduces infarct size in a rat model of ischemia-reperfusion injury.^{31,32} Therefore, it is quite possible that the cardioprotective effect of TAN-67 is mediated via central δ_1 OR activation in vivo. Our in vivo data in the present study are not consistent with results of our prior in vitro investigations.^{33,34} In those studies, we demonstrated that early pretreatment with DPDPE (154 nmol/l) or TAN-67 (178 nmol/l) increased the tolerance of the isolated perfused rat heart to global ischemia and reperfusion.^{33,34} We hypothesize that there are as yet undetermined endogenous factors that can counteract the infarct-reducing effect of δ_1 OR agonists in vivo. Consequently, very high doses of these opioids are required to elevate cardiac tolerance to ischemia and reperfusion injury in vivo.

Pretreatment with the selective κ_1 OR agonist (–)-U-50488 did not alter the IS/AAR ratio. Our findings contradict data of other investigators,^{9,10} who found that U-50488 exhibited an infarct-sparing effect during coronary artery occlusion and reperfusion in rats. Although the exact reason for the diversity between our results and results of other investigators is unknown, we propose that it may be due to the different anesthetics used. We used α -chloralose, but Peart and coworkers used thiobutabarbital,⁹ and Cheng and coworkers used pentobarbital.¹⁰ It is possible that (–)-U-50488 exhibits infarct-reducing effect in rats anesthetized by barbiturates but is ineffective in rats anesthetized by α -chloralose. In support of this, some studies suggest that the molecular mechanism of ischemic preconditioning may be dependent on anesthetics used.³⁵ Prior to this, in 1986, Sander et al. found that cardiovascular effects of met-enkephalin were different in dogs anesthetized by α -chloralose or pentobarbital.³⁶ In 1994, Haessler et al. found that the infarct-sparing effect of ischemic preconditioning is dependent on the anesthetics used.³⁷ Ischemic preconditioning exhibited the strongest infarct-reducing effect in rats anesthetized by pentobarbitone.³⁷

Our study also indicated that the selective κ_2 OR agonist GR-89696 did not alter the IS/AAR ratio. Our in vivo ischemia data are in contrast with our prior findings of a cardioprotective effect of U-50488 (100 nmol/l) during global ischemia and reperfusion of the isolated rat heart.³⁸ Therefore, we cannot discount the possibility of the existence of endogenous factors that prevented the cardioprotective properties of κ OR agonists in vivo.

LIMITATIONS

There are several limitations of this study that should be noted. The lack of cardioprotective effect of the κ_1 opioid, U-50488, and κ_2 opioid GR-89696 at doses tested may be attributed to the use of α -chloralose as the anesthetic rather than the barbiturates described in studies by others. This could be resolved in future studies by comparing α -chloralose- versus barbiturate-induced anesthesia groups at the previously used dose ranges. Higher dose ranges for the δ_2 specific opioids, Deltorphin-II, Deltorphin-D_{variant}, and Deltorphin-E, might provide ischemic cardioprotection. Prior studies by other investigators indicate that Deltorphin-D_{variant} can provide ischemic cardioprotection in an intact pig heart model at 1.0 mg/kg dose,⁵ and that Deltorphin-E at dose range of 2.9 to 5.5 mg/kg can provide ischemic protection from severe hemorrhage in a rat model.³⁹ Since Deltorphin-II is a rather large molecule having a molecular weight of 783, it is unlikely that the cardioprotective effect observed in this study was attributed to activation of central opioid receptors after passage through the blood-brain barrier. Central OR activation by Deltorphin-II can only be ruled out by using ³H-labeled Deltorphin-II, and monitoring passage through the blood-brain barrier.

CONCLUSIONS

We present evidence that Deltorphin II induces infarct-reducing effects at the dose tested in this in vivo animal model of ischemia and reperfusion. Other opioid receptor agonists (Nociceptin, DAMGO, Dermorphin-H, DPDPE, Deltorphin-D, Deltorphin-E, GR-89696, and (–)-U-50488) did not exhibit an infarct-sparing effect at the doses tested. Overall, these results support our hypothesis that opioid mediated cardioprotection is receptor and agonist dependent.

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Table 1

Hemodynamic Parameters of Experimental Groups

Group	n	Baseline			45 min - Ischemia			2h - Reperfusion		
		Heart Rate	MAP	RPP	Heart Rate	MAP	RPP	Heart Rate	MAP	RPP
Control (vehicle)	19	345 ± 48	118 ± 39	41 ± 9	321 ± 68	125 ± 41	41 ± 8	311 ± 43	94 ± 31	34 ± 9
Control (saline)	16	340 ± 50	116 ± 38	40 ± 10	326 ± 66	126 ± 42	41 ± 9	310 ± 44	98 ± 32	35 ± 9
DAMGO 0.077 mg/kg	15	342 ± 52	114 ± 36	39 ± 8	318 ± 63	121 ± 38	38 ± 7	308 ± 47	91 ± 29	29 ± 7
DAMGO 0.77 mg/kg	14	346 ± 42	122 ± 25	42 ± 9	322 ± 44	132 ± 26	41 ± 8	311 ± 38	97 ± 20	32 ± 8
Dermorphin-H 0.23 mg/kg	14	358 ± 78	108 ± 22	38 ± 7	333 ± 38	117 ± 23	38 ± 6	322 ± 70	86 ± 18	31 ± 6
DPDPE 0.097 mg/kg	16	344 ± 81	105 ± 31	36 ± 6	320 ± 54	111 ± 33	37 ± 6	310 ± 73	84 ± 25	29 ± 6
DPDPE 0.97 mg/kg	14	327 ± 37	107 ± 32	35 ± 6	304 ± 56	113 ± 34	36 ± 6	294 ± 33	85 ± 26	29 ± 5
Deltorphin-Dvar 0.3 mg/kg	15	327 ± 89	94 ± 21	31 ± 5	304 ± 36	102 ± 22	30 ± 6	294 ± 80	75 ± 17	27 ± 6
Deltorphin-E 0.18 mg/kg	14	325 ± 55	101 ± 20	33 ± 8	302 ± 35	109 ± 21	32 ± 7	293 ± 50	80 ± 16	28 ± 7
Deltorphin II 0.12 mg/kg	16	334 ± 78	105 ± 31	35 ± 6	310 ± 54	112 ± 33	35 ± 6	301 ± 70	84 ± 25	27 ± 7
GR-89696 0.08 mg/kg	14	312 ± 64	110 ± 29	34 ± 7	290 ± 50	118 ± 31	35 ± 8	281 ± 57	88 ± 23	28 ± 6
GR-89696 0.8 mg/kg	15	315 ± 42	118 ± 34	37 ± 7	292 ± 59	125 ± 36	37 ± 7	283 ± 37	94 ± 27	32 ± 6
U-50488 0.1 mg/kg	14	309 ± 53	116 ± 32	36 ± 9	287 ± 56	123 ± 34	35 ± 8	278 ± 47	92 ± 26	29 ± 8
U-50488 0.6 mg/kg	14	295 ± 48	119 ± 36	35 ± 9	274 ± 63	126 ± 38	36 ± 7	265 ± 43	95 ± 28	28 ± 8
U-50488 1 mg/kg	14	284 ± 37 *	113 ± 24	32 ± 7 *	264 ± 42 *	121 ± 25	33 ± 6 *	285 ± 33	90 ± 19	26 ± 6
Noiceptin 0.4 mg/kg	15	341 ± 50	116 ± 37	40 ± 10	325 ± 65	125 ± 41	41 ± 9	310 ± 42	95 ± 32	34 ± 9
Noiceptin 2.7 mg/kg	15	354 ± 54	105 ± 37	37 ± 6	329 ± 64	113 ± 39	38 ± 7	318 ± 48	84 ± 29	27 ± 6

* p < 0.01 vs. control at each time point.

Table 2

Effect of 150 nmol/kg opioid receptor agonists on the IS/AAR ratio

Protocol	n	LV, mg	AAR, mg	IS, mg	AAR/LV, %	IS/AAR, %
Control (vehicle)	19	504.5 ± 25.7	208.3 ± 25.3	136.2 ± 26.2	41.3 ± 16.6	65.4 ± 11.3
Control (saline)	16	505.2 ± 24.8	206.2 ± 24.1	134.4 ± 25.1	40.8 ± 15.9	65.2 ± 10.9
DAMGO	15	508.3 ± 23.6	212.4 ± 23.6	139.7 ± 30.6	41.8 ± 16.7	65.7 ± 15.9
Dermorphin H	14	494.8 ± 21.7	213.7 ± 22.1	138.8 ± 30.3	43.2 ± 14.6	64.9 ± 14.6
Deltorphin D _{variant}	15	501.6 ± 24.0	209.9 ± 22.2	137.7 ± 30.2	41.8 ± 15.9	65.6 ± 14.3
Deltorphin E	14	503.7 ± 24.3	213.5 ± 23.2	140.1 ± 30.7	42.4 ± 16.8	65.6 ± 16.1
DPDPE	16	499.2 ± 23.6	211.2 ± 19.6	138.9 ± 31.2	42.3 ± 14.4	65.7 ± 14.4
Deltorphin II	16	498.6 ± 22.0	211.3 ± 17.6	106.9 ± 29.2 *	42.4 ± 16.8	50.6 ± 12.4 *
Nociceptin	15	504.2 ± 25.6	210.8 ± 21.3	141.1 ± 30.2	41.8 ± 16.3	66.9 ± 14.7

Values are mean ± SD.

* p<0.01 vs. the control group.

n = no. of animals; LV = left ventricle; AAR = area at risk; IS, infarct size.

Control (vehicle) is 20% hydroxypropyl-beta-cyclodextrin solution.

Opioid doses: DAMGO (0.077 mg/kg), Dermorphin-H (0.23 mg/kg), Deltorphin-D_{variant} (0.3 mg/kg), Deltorphin-E (0.18 mg/kg), DPDPE (0.097 mg/kg), Deltorphin-II (0.12 mg/kg) and Nociceptin (Orphanin FQ) (0.4 mg/kg).

Table 3

Effect of 1500 nmol/kg doses of μ , δ_1 and ORL1 receptor agonists on the infarct size/area at risk ratio.

Protocol	n	LV, mg	AAR, mg	IS, mg	AAR/LV, %	IS/AAR, %
Control (saline)	16	497.6 $\pm$ 20.3	204.9 $\pm$ 12.6	123.5 $\pm$ 18.6	41.2 $\pm$ 11.0	60.6 $\pm$ 10.4
DAMGO	14	497.0 $\pm$ 16.5	202.7 $\pm$ 7.8	120.9 $\pm$ 26.2	41.8 $\pm$ 13.5	59.7 $\pm$ 13.1
DPDPE	14	499.4 $\pm$ 15.3	204.1 $\pm$ 7.1	121.9 $\pm$ 25.8	40.9 $\pm$ 13.8	59.9 $\pm$ 13.1
Nociceptin	15	500.9 $\pm$ 19.8	205.2 $\pm$ 7.0	122.9 $\pm$ 20.5	41.0 $\pm$ 11.2	59.8 $\pm$ 10.1

Values are mean $\pm$ SD.

n = no. of animals; LV = left ventricle; AAR = area at risk; IS = infarct size.

Opioid doses: DAMGO (0.77 mg/kg), DPDPE (0.97 mg/kg), Nociceptin (2.7 mg/kg).

The experimental group results were not statistically different when compared to the Control (vehicle) group.

Table 4

Effect of increasing doses of κ opioid receptor agonists on the infarct size/area at risk ratio.

Protocol	n	LV, mg	AAR, mg	IS, mg	AAR/LV, %	IS/AAR, %
Control (saline)	16	498.2 $\pm$ 20.4	205.1 $\pm$ 12.4	122.8 $\pm$ 21.0	41.1 $\pm$ 11.9	60.2 $\pm$ 11.3
(-)-U-50488 (0.1 mg/kg)	14	500.6 $\pm$ 13.1	203.4 $\pm$ 8.6	126.2 $\pm$ 33.0	40.6 $\pm$ 8.6	61.9 $\pm$ 17.6
(-)-U-50488 (0.6 mg/kg)	14	491.8 $\pm$ 32.9	204.7 $\pm$ 7.9	121.6 $\pm$ 19.4	41.6 $\pm$ 15.3	59.4 $\pm$ 9.7
(-)-U-50488 (1 mg/kg)	14	497.3 $\pm$ 19.1	203.3 $\pm$ 10.8	123.9 $\pm$ 22.1	40.8 $\pm$ 10.1	61.2 $\pm$ 12.0
GR-89696 (0.08 mg/kg)	14	495.8 $\pm$ 19.5	205.3 $\pm$ 7.1	124.4 $\pm$ 27.7	41.4 $\pm$ 9.0	60.7 $\pm$ 13.8
GR-89696 (0.8 mg/kg)	15	495.3 $\pm$ 19.8	201.6 $\pm$ 7.4	117.7 $\pm$ 33.0	40.7 $\pm$ 15.5	58.4 $\pm$ 15.9

Values are mean $\pm$ SD. n = no. of animals; LV = left ventricle; AAR = area at risk; IS = infarct size.

The experimental group results were not statistically different when compared to the Control (vehicle) group.